CHAPTER 2

THE DIOXIN ASSAY

PARTICIPANTS SELECTED FOR DIOXIN MEASUREMENT

Participants at the 1992 physical examination eligible to have blood drawn for the dioxin assay were assigned to one of three categories: previous participants with a quantifiable dioxin result who were selected for an additional blood draw to advance pharmacokinetic studies (1), previous participants returning to the 1992 physical examination with no prior dioxin blood draw or no previously quantifiable dioxin results, and first-time participants. Table 2-1 shows the number of participants eligible for the 1992 dioxin blood draw belonging to each category by exposure group (Ranch Hand, Comparison). Table 2-1 also gives the number of actual dioxin assay results observed by participant category and exposure group.

A total of 835 participants in the 1992 examination were invited for the blood draw. Blood samples from 62 participants were unavailable for analysis at Centers for Disease Control (CDC). Table 2-2 displays the reasons for this reduction. Five participants not meeting eligibility criteria had blood drawn inadvertently. Sixteen participants were medically deferred, 34 refused, 2 had unsuccessful blood collection, 2 eligible participants were inadvertently omitted, and 3 participants were excluded when their unit bags broke during processing. Samples for the remaining 773 participants were shipped to CDC.

SAMPLE ACQUISITION

Blood was drawn from volunteers for the serum dioxin assay on the morning of the second day of the 1992 physical examination cycle. The participants fasted after midnight (water was allowed); samples were drawn with a 15-gauge needle into a blood pack unit without anticoagulant. The blood pack units had been previously tested by CDC and found to be free of dioxin contamination. Participants selected for the immunology studies had 250 ml of blood drawn; all others had 350 ml of blood drawn. After the drawing, the bags were clamped, labeled, placed upright at room temperature, and the samples allowed to clot for 7 hours.

The clotted samples were centrifuged for 15 minutes at 4,500 RPM at a temperature of 4°C to 10°C. The serum was then transferred from the spun unit bag to transfer packs (also dioxin-free) by a plasma extractor. The transfer packs then were spun for 15 minutes at 4,500 RPM. The serum was then placed into four Wheaton bottles: two 4-ounce bottles for the serum dioxin analysis, a 5 ml bottle for the lipid profile, and a 10 ml bottle for reserve serum. Samples were cataloged and stored at -20°C or less until shipment. Appendix A-1 contains the detailed procedures used by the Scripps Clinic and Research Foundation (SCRF) for the dioxin blood collection and processing. Frozen samples were packed in dry ice in styrofoam boxes and shipped twice weekly from SCRF, La Jolla, California, to Brooks Air Force Base, Texas. At Brooks Air Force Base, inventory was taken and the specimens were stored at -70°C until shipment to CDC. All samples were coded so that the group status of each specimen (Ranch Hand, Comparison) was unknown to the CDC staff.

Table 2-1.
Participants Eligible for the 1992 Dioxin Blood Draw

Participant Category	Number Eligible			Number Results		
	RH	C	Total	RH	C	Total
Returning participants with previous quantifiable dioxin result selected for another blood draw	341	47	388	329	44	373
Returning participants with no previous dioxin blood draw or no previous quantifiable dioxin result	103	211	314	91	194	285
Participants new to study	38	90	128	35	80	115
Total	482	348	830	455	318	773

RH = Ranch Hand.
C = Comparison.

Table 2-2.
Participants Invited for the 1992 Dioxin Blood Draw and Reasons for Participant Sample Exclusions

Distribution of Sample Exclusion	Ranch Hand	Comparison	Total
Total Invited	483	352	835
Less:			
• Inadvertent Additional Draws (Did not meet Eligibility Criteria)	(1)	(4)	(5)
Total Selected for Blood Draw	482	348	830
Less:			
• Medically Deferred	(8)	(8)	(16)
• Refused	(16)	(18)	(34)
• Attempted, Unsuccessful	(1)	(1)	(2)
• Inadvertent Omissions	(1)	(1)	(2)
• Bag Broke	(1)	(2)	(3)
Total Specimens Sent to CDC	455	318	773

ANALYTICAL METHOD

The serum samples were analyzed for dioxin in groupings consisting of a method blank, three unknown samples, and a quality control (QC) pool sample (2,3). Cholesterol esters, triglycerides, and high-density lipoprotein (HDL) cholesterol were determined in duplicate by standard methods. Total phospholipids were determined in duplicate by modifying the Folch et al. procedure (4,5). Fresh cholesterol was determined in duplicate by an enzymatic method (6). For each analysis, the mean result of duplicate analyses was used to calculate the concentrations of total lipids using the summation method (7), low-density lipoprotein cholesterol, and very low-density lipoprotein cholesterol (8).

QUALITY CONTROL

Quality assurance (QA) was maintained with matrix-based materials well-characterized for dioxin concentration and isotope ratios to ensure that the analytical system was in control. QC charts were maintained for each of these materials (five serum pools). The concentration in the QC sample from each analytical run was required to be within established 99-percent confidence limits (9,10). The unlabeled and carbon-13 labeled internal standard isotope ratios were required to be within 95-percent confidence limits. All analytical runs for the dioxin and lipid measurements were in control. No dioxin was detected in the blanks (on-column injection of 100 femtograms from a standard solution produces detectable signals greater than three times the background noise).

DATA DESCRIPTION

CDC delivered whole-weight and lipid-adjusted dioxin concentrations to the Air Force, together with the total sample weight, weights of lipid fractions, total lipid weight, detection limit, quantitation limit, and all associated QC information, including results from blank samples. The lipid-adjusted dioxin concentration is a calculated quantity based on the whole-weight dioxin concentration and the total lipid weight. Details of the calculation are discussed subsequently in this chapter.

The analyses in this report are based in part on 522 of the total 773 assay results. These 522 results were available at the commencement of the statistical analyses, and the additional 251 dioxin assay results were received after the statistical analysis began. Table 2-3 provides the results of the 1992 physical examination blood draws by exposure group and result comment (i.e., the notes on dioxin result). This table is divided into two descriptive sections: the 522 results used in the analyses in this report and the 251 assay results received after the commencement of the statistical analyses. The third section of the table provides totals. Additional statistics on these 251 assay results are given later in this chapter.

The dioxin data base is a combination of the dioxin assay results from the 1987 and 1992 examination. Figure 2-1 shows the number of dioxin blood draw results by year, and exemplifies the high percentage of study participants who have dioxin measurements. Of the 2,233 fully compliant participants, 1,970 (88.2%) had blood drawn in 1987; 545 of these 1,970 participants who had blood drawn in 1987 also had blood drawn in 1992. Figure 2-2

Table 2-3.
Result Comments for 1992 Blood Draw Assays

Result Comment	Ranch Hand	Comparison	Total
	Assays Available Before the Commencement of the Statistical Analysis (n=522)		
G	366	92	458
GND	3	15	18
GNQ	2	2	4
NR	32	10	42
Total	403	119	522
	Assays Available After the Commencement of the Statistical Analysis (n=251)		
G	46	149	195
GND	5	31	36
GNQ	1	13	14
NR	0	6	6
Total	52	199	251
	Total of 1992 Blood Draw Assays (n=773)		
G	412	241	653
GND	8	46	54
GNQ	3	15	18
NR	32	16	48
Total	455	318	773

G = Good result.
GND = Good result, below limit of detection.
GNQ = Good result, below limit of quantitation.
NR = No result.

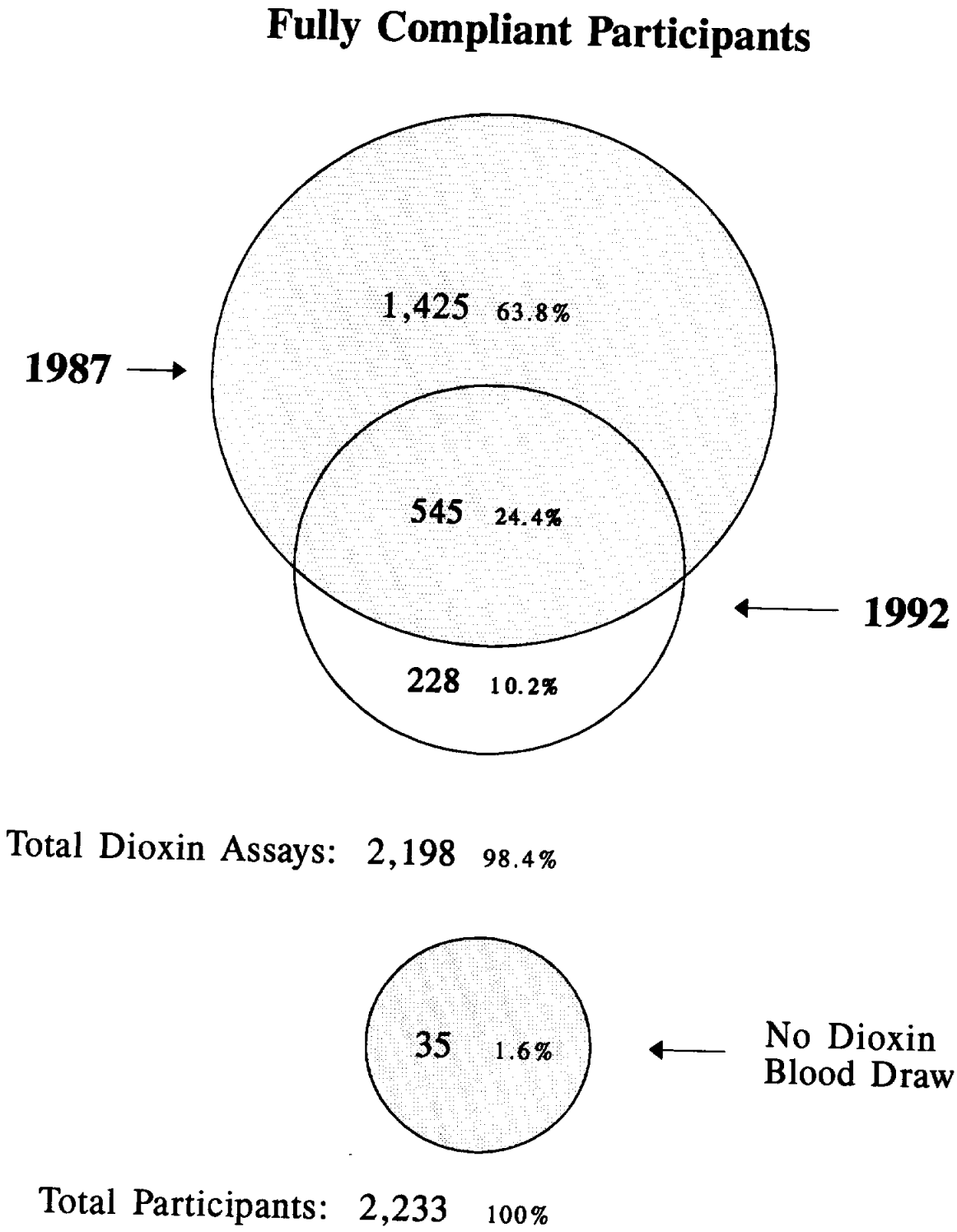


Figure 2-1. Dioxin Results for 2,233 Fully Compliant Participants at the 1992 Physical Examination

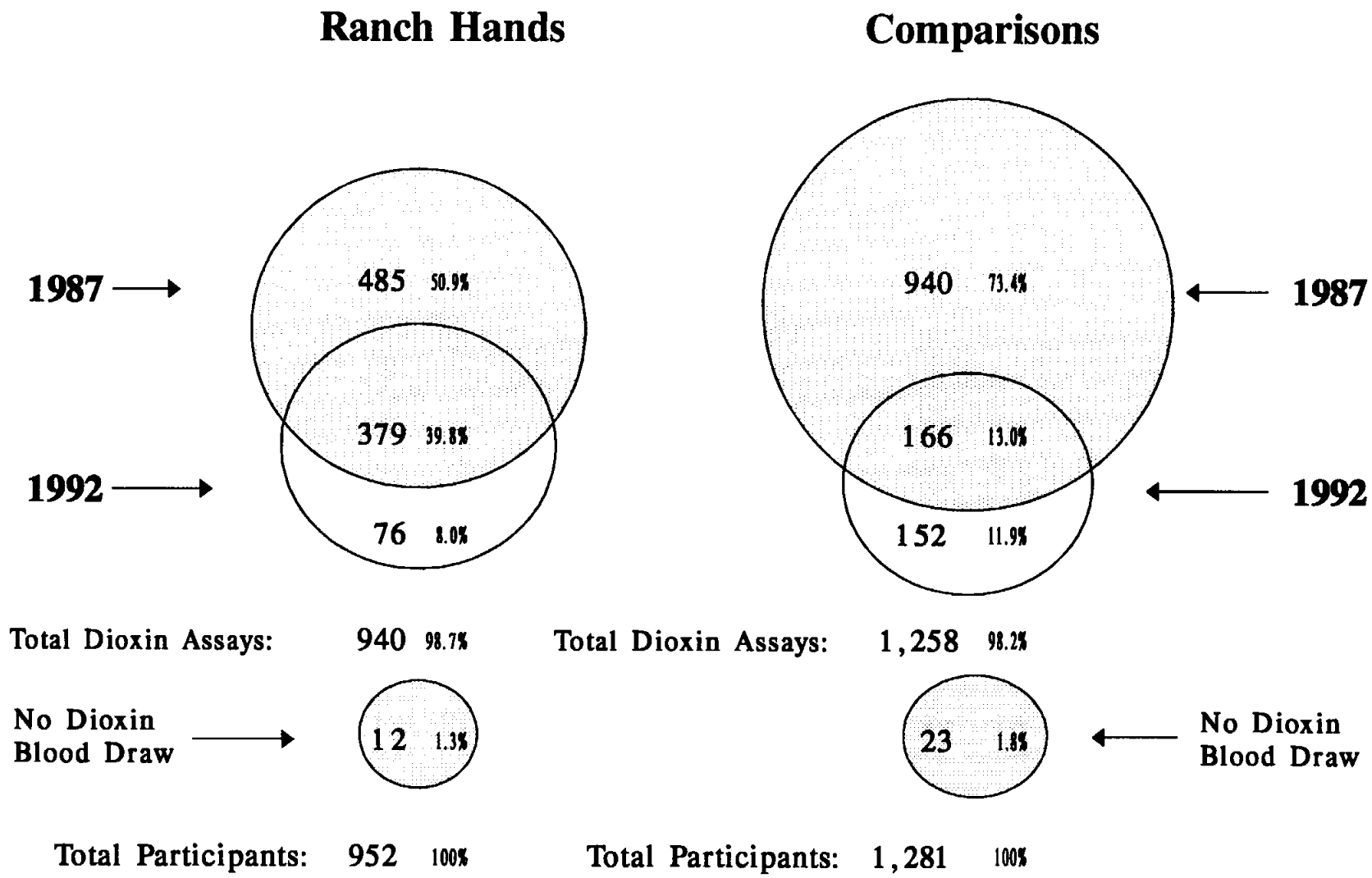


Figure 2-2. Dioxin Results for 952 Fully Compliant Ranch Hands and 1,281 Fully Compliant Comparisons at the 1992 Physical Examination

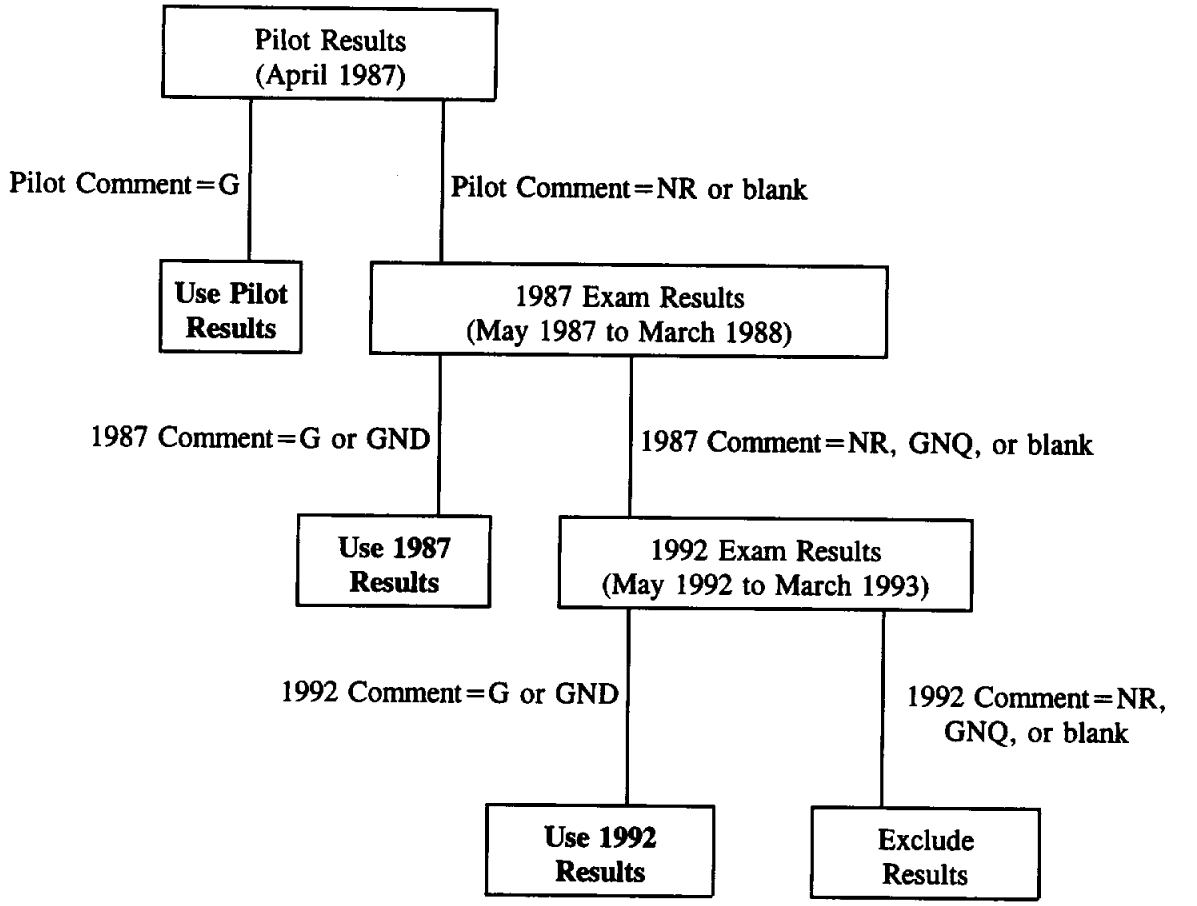
shows the number of dioxin blood draws by both year and exposure group. Almost 70 percent of those participants assayed twice were Ranch Hands (379 out of 545).

Participants may have been assayed for any combination of three events: the pilot study conducted in April 1987 (9), the 1987 followup examination (May 1987 to March 1988), or the 1992 followup examination (May 1992 to March 1993). The majority of participants had an assay in 1987, either in conjunction with the pilot study or the 1987 followup examination. Consequently, 1987 was designated as the reference point for current dioxin assays. When a participant had multiple assay results, first priority was given to the 1987 pilot-study dioxin results, second priority was given to results derived from serum collected at the 1987 physical examination, and third priority was given to the 1992 results. Figure 2-3 outlines this decision process. If a quantifiable pilot-study assay was available, it was used. Otherwise, a 1987 assay (if available and quantifiable) or a 1992 measurement was used. For use in models based on current dioxin, if the 1992 measurement was used (n=83 for samples used for the statistical analyses), the level was extrapolated to 1987 levels when the 1992 dioxin concentration surpassed 10 ppt (n=34). These extrapolated lipid-adjusted dioxin values were calculated using a first-order decay model with a half-life of 7.1 years and a background level of 4 ppt. Levels at or below 10 ppt were not extrapolated because the first-order decay model was not considered to be valid at background levels (lipid-adjusted current dioxin levels $\leq$ 10 ppt). Details on the extrapolation method are given in Chapter 7, Statistical Methods.

Of the 2,233 fully compliant participants at the 1992 physical examination, 952 were Ranch Hands and 1,281 were Comparisons. Of the 2,233 participants, 35 never had blood drawn for a dioxin assay (see Figure 2-1). Forty-four participants had missing dioxin results (result comment=NR) or nonquantifiable dioxin results (result comment=GNQ). A total of 2,154 participants, consisting of 930 Ranch Hands and 1,224 Comparisons, had quantifiable dioxin measurements. Of these 2,154 participants, 1,980 were available at the commencement of the statistical analyses (894 Ranch Hands and 1,086 Comparisons). The remaining 174 assays (36 Ranch Hands and 138 Comparisons) were received after the start of the statistical analysis. Table 2-4 summarizes the sample-size reduction by exposure group and further classifies the 2,154 participants according to their availability for statistical analysis. Participants with missing or nonquantifiable dioxin results are cross-classified in Table 2-5 by result comment and exposure group.

Lipid-Adjusted and Whole-Weight Current Dioxin Measurements

Serum dioxin is defined as the serum concentration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Its relationship with dioxin concentrations in other compartments, such as adipose tissue, is a subject of continuing research. Serum dioxin, as analyzed in this report, can be expressed as a lipid-adjusted or a whole-weight measurement. The lipid-adjusted dioxin measurement, also called "current dioxin body burden," is a derived quantity calculated from the formula ppt = ppq·102.6/W, where ppt is the lipid-adjusted concentration, ppq is the actual weight of dioxin in the sample (also known as whole-weight dioxin) in femtograms, 102.6 corrects for the average density of serum, and W is the total lipid weight of the sample (10). The correlation between the serum lipid-adjusted concentration and adipose tissue lipid-adjusted concentration of dioxin has been observed to be 0.98 in 50 persons from Missouri (11). Using the same data, Patterson et al. calculated the



G = Good result.
GND = Good result, below limit of detection.
GNQ = Good result, below limit of quantification.
NR = No result.

Figure 2-3. Decision Process for Determination of Dioxin Results for Analysis

Table 2-4.
Dioxin Blood Draw Results

Summary of Sample-Size Reduction and Participant Availability	Ranch Hand	Comparison	Total
Fully Compliant to 1992 Physical Examination	952	1,281	2,233
Less: No Blood Draw for Dioxin at any Physical Examination	(12)	(23)	(35)
Participants Fully Compliant to 1992 Physical Examination with a Dioxin Assay	940	1,258	2,198
Less: Missing or Nonquantifiable Results (Good result, but below limit of quantitation or No Result)	(10)	(34)	(44)
Participants with Quantifiable Dioxin Results	930	1,224	2,154
Available Before the Commencement of the Statistical Analysis	894	1,086	1,980
Available After the Commencement of the Statistical Analysis	36	138	174

Table 2-5.
Dioxin Blood Draw Results with Missing or Nonquantifiable Results

Result Comment				
1987 Assay	1992 Assay	Ranch Hand	Comparison	Total
	GNQ	1	7	8
	NR	4	4	8
GNQ		1	2	3
GNQ	GNQ	1	7	8
GNQ	NR	0	7	7
NR		2	2	4
NR	GNQ	0	1	1
NR	NR	1	4	5
	Total	10	34	44

GNQ = Good result, below level of quantification.
NR = No result.

partitioning ratio of dioxin between adipose tissue and serum on a lipid-adjusted basis as 1.09 (95% C.I. = [0.97, 1.21]). On the basis of these data, a one-to-one partitioning ratio of dioxin between lipids in adipose tissue and lipids in serum cannot be excluded. Measurements of dioxin in adipose tissue generally have been accepted as representing the body-burden concentration of dioxin. The high correlation between serum dioxin levels and adipose-tissue dioxin levels in their study suggests that serum dioxin is also a valid measurement of dioxin body burden.

Figures 2-4 and 2-5 show the distribution of serum lipid-adjusted current dioxin for the 894 Ranch Hands and 1,086 Comparisons whose results were used in analyses of current dioxin versus health in this report. The 95th, 98th, and 99th percentiles of serum lipid-adjusted current dioxin distribution for Ranch Hands were 101.7, 156.2, and 200.5 ppt respectively; percentiles for the corresponding Comparisons were 8.5, 10.2, and 13.5 ppt. Figure 2-6 compares distributions of the logarithm (base 2) of serum lipid-adjusted dioxin concentrations for Ranch Hands and Comparisons.

Table 2-6 summarizes, by military occupation and exposure group, the serum lipid-adjusted dioxin results among the 894 Ranch Hands and 1,086 Comparisons whose results were used in analyses of dioxin versus health in this report. Serum whole-weight dioxin results are presented in Table 2-7.

Dioxin Results Provided After the Commencement of the Statistical Analyses

CDC provided the remaining 251 dioxin results after the commencement of the statistical analyses (see Table 2-3). Of these 251 additional results, 52 belonged to Ranch Hands and 199 belonged to Comparisons. Of the 52 additional Ranch Hand results, 51 were quantifiable (result comment=G or GND) and one was nonquantifiable (result comment= GNQ). The median current dioxin level for these 51 Ranch Hands was 5.1 ppt. Ranch Hand dioxin levels ranged between 0 ppt and 110.7 ppt; the first and third quartiles were 3.2 ppt and 8.8 ppt. All 51 quantifiable results fell between the minimum and maximum observed for the 894 Ranch Hands whose data were used in this report. Of the 199 additional Comparison results, 180 were quantifiable (result comment=G or GND) and 19 were nonquantifiable (13 had a result comment of GNQ, and 6 had a result comment of NR). For the 180 quantifiable Comparison results, the median was 3.1 ppt, the range was between 0 ppt and 13.8 ppt, and the first and third quartiles were 2.1 ppt and 4.7 ppt. All 180 quantifiable results fell between the minimum and maximum observed for the 1,086 Comparison results used in this report.

Of the 51 additional quantifiable Ranch Hand results, 15 belonged to Ranch Hands who had a previous quantifiable 1987 dioxin result. Similarly, of the 180 additional quantifiable Comparison results, 42 belonged to Comparisons who had a previous quantifiable dioxin result; these additional results are included in Tables 2-6 and 2-7. Inclusion of the 15 Ranch Hand and 42 Comparison 1992 assay results (had they been received before the commencement of the statistical analysis) would not alter the analysis because, when a participant had multiple assays, priority was given to 1987 results. The remaining 174 (36 Ranch Hand and 138 Comparison) quantifiable results were not included in analyses of dioxin versus health in this report; these individuals were included in the overall group contrasts (Ranch Hand versus

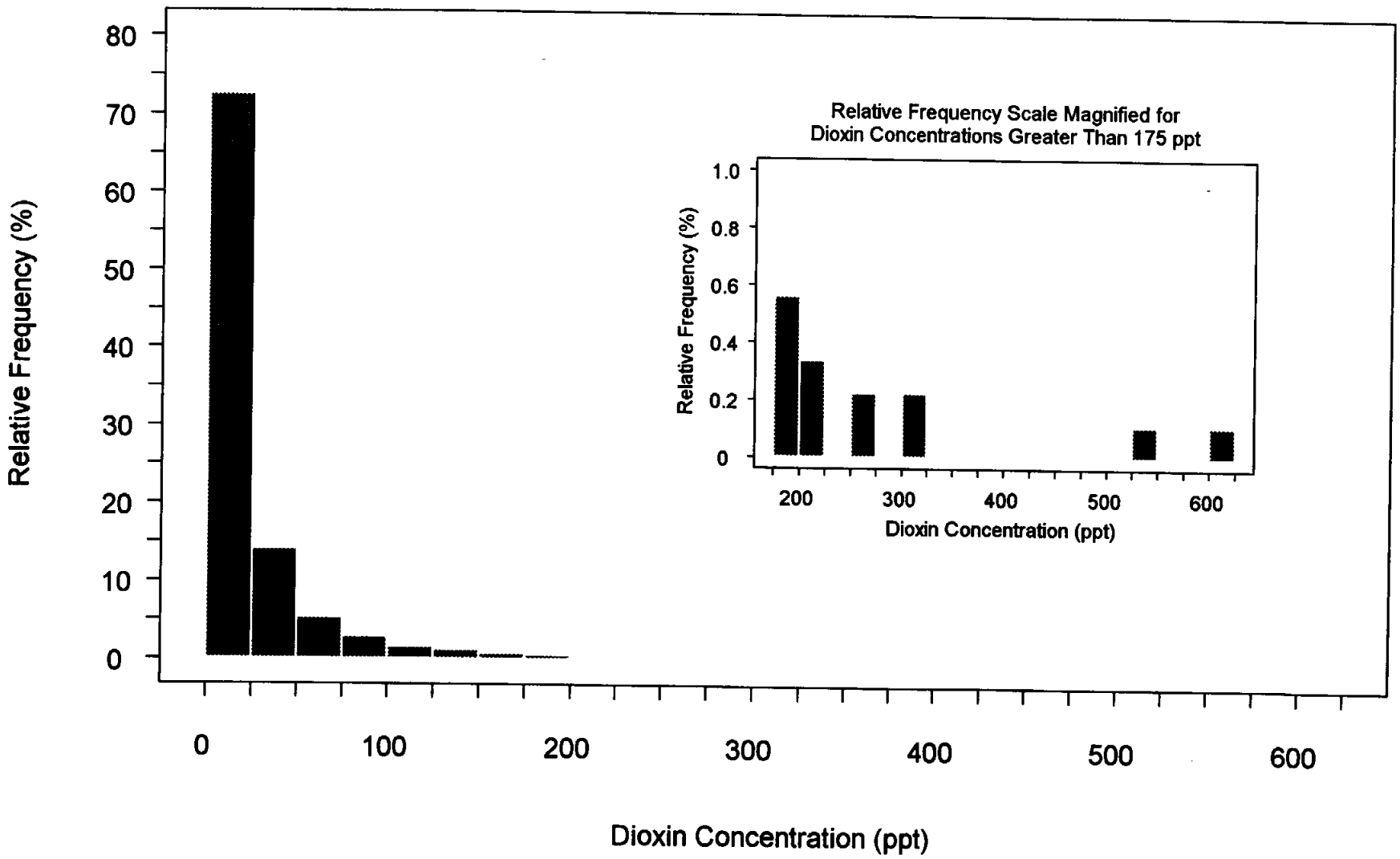


Figure 2-4. Relative Frequency Distribution of Lipid-Adjusted Dioxin Concentrations for 894 Ranch Hands

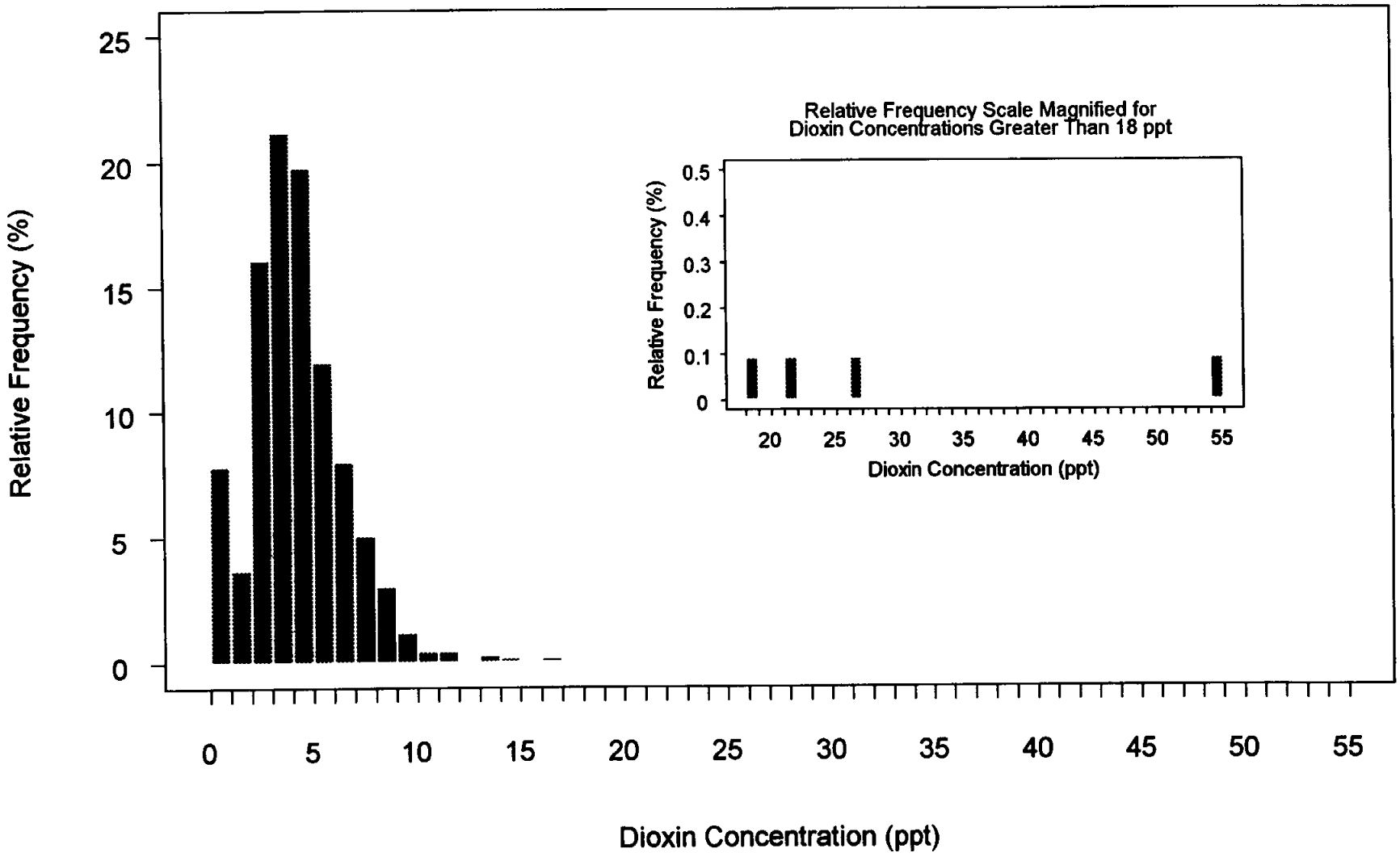


Figure 2-5. Relative Frequency Distribution of Lipid-Adjusted Dioxin Concentrations for 1,086 Comparisons

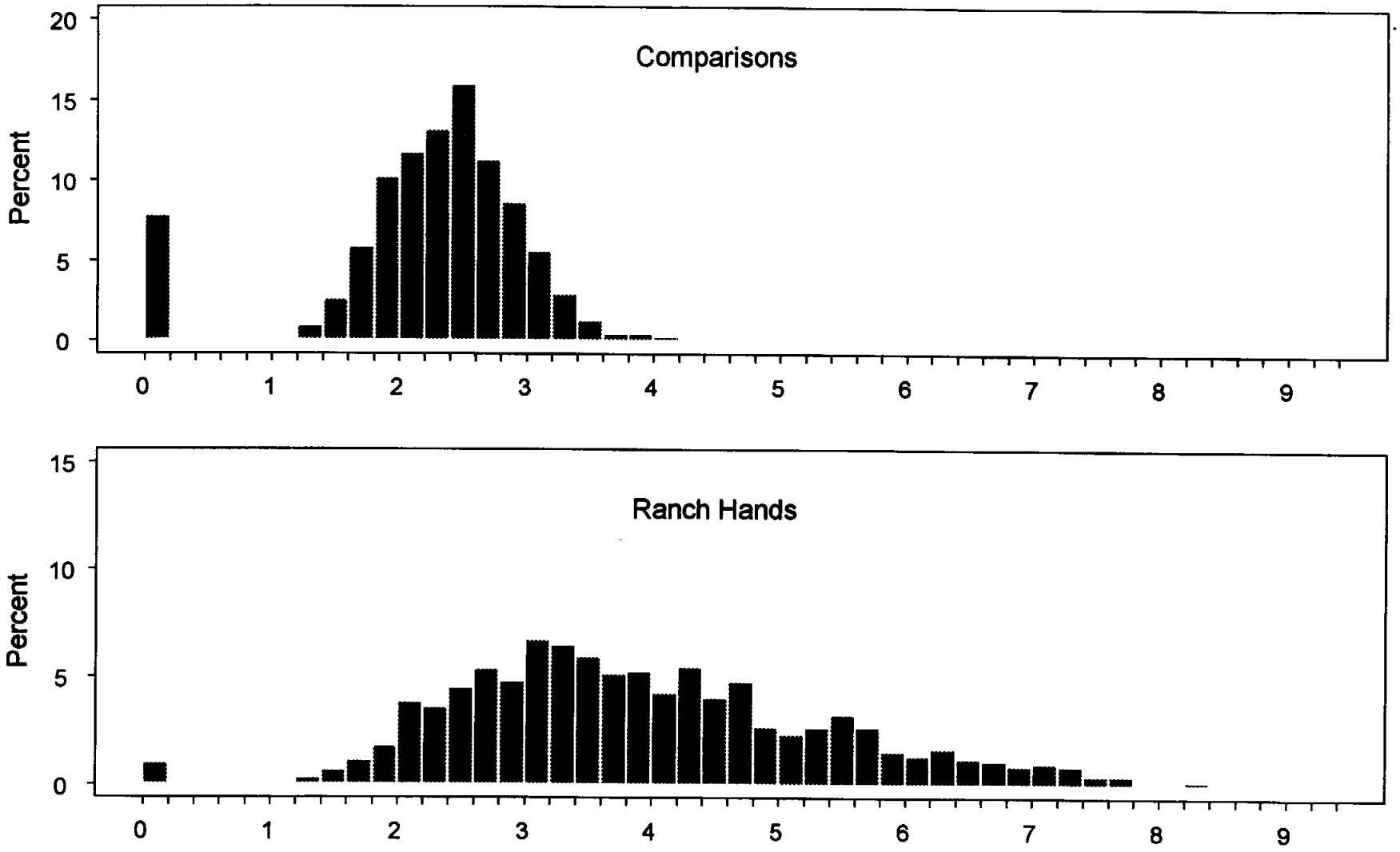


Figure 2-6. Relative Frequency Distribution of the Logarithm (Base 2) of Lipid-Adjusted Dioxin Concentrations

Table 2-6.
Lipid-Adjusted Dioxin Result Summary of 894 Ranch Hands and 1,086 Comparisons Used in the Statistical Analysis

	Ranch Hand			Comparison		
Military Occupation	n	Median	Range	n	Median	Range
Officer	348	7.7	0-36.0	420	4.4	0-18.5
Enlisted Flyer	150	17.8	0-195.5	174	4.0	0-12.8
Enlisted Groundcrew	396	24.1	0-617.8	492	4.0	0-54.8
Total	894	12.5	0-617.8	1,086	4.1	0-54.8

Table 2-7.
Whole-Weight Dioxin Result Summary of 894 Ranch Hands and 1,086 Comparisons Used in the Statistical Analysis

	Ranch Hand			Comparison		
Military Occupation	n	Median	Range	n	Median	Range
Officer	348	45.0	0-332.0	420	25.0	0-158
Enlisted Flyer	150	98.4	0-1,537.8	174	25.3	0-181
Enlisted Groundcrew	396	148.0	0-5,433.0	492	22.0	0-318
Total	894	74.8	0-5,433.0	1,086	24.0	0-318

Comparison), however. Additional analyses of malignant systemic cancer and serum insulin were subsequently performed with the inclusion of the 174 dioxin results, to determine whether the inclusion of these dioxin results would alter the conclusions. Appendix A-2 contains the results of the additional analyses.

SUMMARY

In summary, 91 percent of the 1,281 fully compliant Comparisons and 96 percent of the 952 fully compliant Ranch Hands at the 1992 physical examination had dioxin assay results. Eighty-five percent of the 1,281 Comparisons and 94 percent of the 952 Ranch Hands had quantifiable results used in the statistical analyses in this report. Additional dioxin results became available after the commencement of the statistical analyses. These additional data were incorporated into several analyses, documented in Appendix A-2, which had little effect on the analysis results provided in this report.

CHAPTER 2
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